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A silent deletion in the β-globin gene cluster

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Abstract

A survey of the γ-globin gene region of over 1000 normal individuals revealed a novel 2.5kb deletion which removes the 5' end of the $^{A}\gamma$-globin gene. Unusually, this deletion in the β-globin gene cluster is not associated with increased fetal haemoglobin production. Sequence analysis of the deletion endpoints revealed no significant homology at the breakpoint and failed to support a role for a proposed recombination hotspot in IVS-2 in the generation of this illegitimate recombination event. The existence of small "silent" deletions in the β-globin gene cluster emphasizes the importance of deletion size in altering expression of the fetal globin genes.

INTRODUCTION

The genes of the β-globin cluster are arranged on the short arm of chromosome 11 in the order in which they are expressed: $\varepsilon\text{-}^{G}\gamma\text{-}^{A}\gamma\text{-}\delta\text{-}\beta$ (1,2). The switch from expression of the fetal γ to the minor and major adult genes, δ and β, has been extensively investigated as a model for studying developmental gene regulation. However, an understanding of the control of this switch is also of considerable clinical importance, because an ability to increase fetal hemoglobin production in sickle cell anaemia and β thalassaemia might considerably alleviate the course of these diseases (3).

Natural deletions in the β gene cluster have been extensively studied in an attempt to define control regions of importance in developmental switching. Over 20 large deletions have now been defined and various models and control regions proposed (reviewed in 1,2). However, all these deletions have been selected for analysis because they produce an abnormal phenotype. It seemed possible that there might also exist smaller deletions within the cluster, without a significantly altered phenotype, that might be equally informative in defining or excluding particular control regions for developmental switching. Unfortunately, finding such silent deletions involves screening of DNA samples from large numbers of normal indiviuals. As part of a survey of globin genes in the Melanesian populations of the Southwest Pacific (4) we have analysed the γ globin gene region of over 1000 individuals. These

populations have a high frequency of single and triplicated γ gene chromosomes and a rare quadruplicated γ gene chromosome (5,6,7). A single individual was found with a small deletion in the region of the γ globin genes who had a normal fetal haemoglobin (Hb F) level. By detailed restriction mapping, cloning and sequence analysis we have defined the end points of this novel deletion which does not interfere with developmental switching.

MATERIALS AND METHODS

Blood samples were collected from healthy individuals on islands throughout Melanesia from Papua New Guinea in the northwest to New Caledonia in the south. DNA was extracted and Southern blotting, using a 3.3 kb Hind III $^{A}\gamma$-globin gene probe and a 1.7 kb Bgl II - Xba I $\psi\beta$ fragment, performed as described (8). HbF levels were measured by standard methods (9). For cloning and sequencing (10), genomic DNA from the deletion heterozygote was digested to completion with Bgl II and enriched for the 10.5 kb deleted allele by 5 to 20% isokinetic sucrose gradient fractionation. This fragment was cloned into the bacteriophage L47.1 at the Bam H1 sites and plaques were screened using the 3.3 kb γ-probe. Positive plaques were rescreened to homogeneity, then DNA was prepared and mapped. Recombinant phage DNA was sequenced using the chemical degradation method of Maxam and Gilbert (11).

RESULTS

Population survey.

1056 individuals were screened for rearrangements in their γ- globin gene regions using the restriction enzyme Bgl II and a 3.3 kb $^{A}\gamma$-gene probe. Normal chromosomes produce a 13 kb band (Fig. 1) and single, triplicated and quadruplicated γ-gene chromosomes were identified by the presence of 8 kb, 18 kb and 23 kb bands, as reported (5). Three individuals, two from Vanuatu and one from New Caledonia, showed two new bands of 9.5 kb and 3.5 kb, which by further multiple enzyme digests were shown to be due to a novel Bgl II site polymorphism just 3' to the $^{G}\gamma$ gene. Additionally, an adult from the island of Pentecost in northern Vanuatu, showed an extra 10.5 kb band on Bgl II digestion (Fig. 2). Two other enzymes which cut outside the γ gene region, Bcl I and Eco RV, also showed extra bands 2.5 kb smaller than normal in this individual (Table 1). This suggested that there was a deletion of 2.5 kb in the γ-gene region of one chromosome and this was confirmed by showing that the new, smaller (12 kb) band found on Eco RV digestion also hybridized to a $\psi\beta$-globin gene probe (Fig. 1).

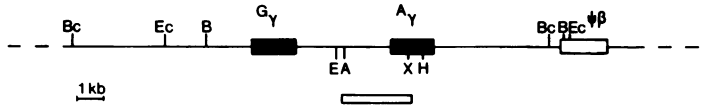

Figure 1

Restriction enzyme map of γ-globin gene region indicating the position of the 2.5 kb deletion (open bar). Sites for restriction enyzmes which cut outside of both γ-genes are shown above the complex:- Bc, Bcl I; Ec, EcoRV; B, Bgl II. The Eco R1 (E), Acc I (A), Xba I (X) and Hpa I (H) sites used to map the end points of the deletion are also indicated.

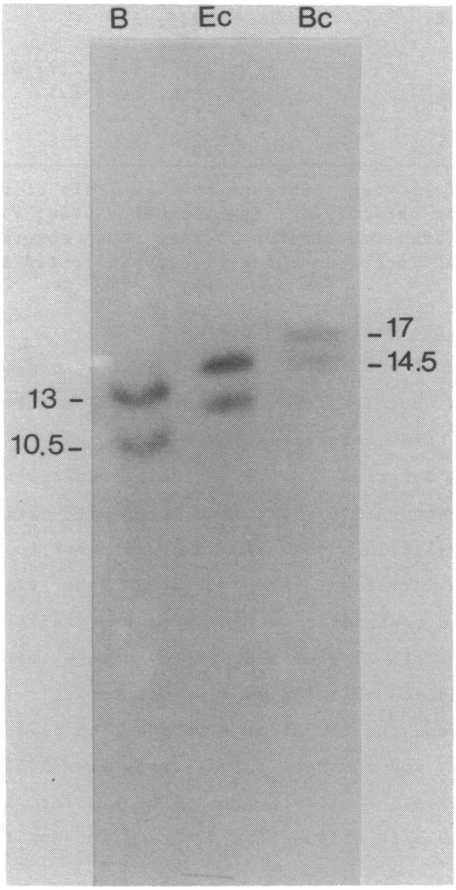

Figure 2

Southern blot band patterns produced by DNA from the deletion heterozygote using Bgl II (B), Eco RV (E) and Bcl I (Bc), following hybridisation with the γ-globin probe.

TABLE I

Southern blot analysis of the deletion heterozygote

Restriction Enzymes	Band sizes (kb)	
Acc I	4.8, 4.4 2.0^{+}, 1.0	
Apa I	~22, 5.0	~25
Bam HI	15.5, 5.0, 2.6	18
Bcl I	17,	14.5
Bgl I	5.0, 3.7, 3.0	5.5
Bgl II	13,	10.5
Bst EII	~30, 5.5, 3.7, 1.2	2.4
Eco RI	7.0, 2.6	
Eco RV	14.5	12
Hinc II	4.0, 2.9, 2.7, 1.0	1.5
Hind III	7.5, 3.3	1.5
Hpa I	23.5, 13.5, 5.0	2.5
Pst I	5.0, 4.0, 2.7^{+}, 0.8	3.3
Sac I	3.6, 2.6, 1.1^{+}	
Sph I	17, 4.4,	1.9
Xba I	7.4, 5.0, 3.8	9.9

Band sizes seen following Southern blot analysis of DNA with a γ-globin probe in the deletion heterozygote from Vanuatu, using various restriction enzymes. Normal bands are listed before the abnormal bands which are underlined. Bands of increased intensity are indicated by an asterisk.

A silent deletion in the β-globin complex.

The Hb F level of the adult in whom this 2.5 kb deletion was identified was 0.2%, well within the normal range. Further haematological data are not yet available for this individual who lives on a remote island. All previously identified deletions in the β-globin complex, with the exception of single $^{A}\gamma$ gene chromosomes (5,6), are associated with variably elevated levels of fetal haemoglobin (1,2). Hence, it was of interest to map precisely the end points of this deletion. The γ-globin gene region has been fully sequenced and detailed restriction enzyme maps are available (12,13). Using a total of 16 enzymes (Table 1) it was possible to show that the 5' end of the $^{A}\gamma$ gene was deleted and that the Gγ gene was intact. The 5' breakpoint was thus shown to be between the Eco RI site at position 5135 of the sequence of Shen et al, 1981 (13), and the Acc I site at position 5455, approximately half way between the γ-genes. Similarly, the 3' breakpoint was in IVS-2 of the $^{A}\gamma$ gene between the Xba I site at position 7743 and the Hpa I site at position 8305.

Cloning and Sequencing of the Deletion Endpoints.

The region shown above to encompass the deleted 2.5 kb includes a run of alternating purine-pyrimidine bases, which has some single-stranded DNA characteristics in supercoiled plasmids, and has been proposed as a "hotspot"

TABLE 2

DNA Sequence at the Deletion Breakpoint

	5305 ↓
Inter -γ region	AAACGTCTTCTGCCTGGCctccattcaaatcataaagg
Deletion chromosome $^{A}\gamma$ IVS-2	AAACGTCTTCTGCCTGGCTTTCATCTTTCCCTCATTTT
	gattccagtagaaagaaCTTTCATCTTTCCCTCATTTT
	7827 ↓

DNA sequence at the breakpoint of the 2.5 kb deletion. The middle line shows the sequence of the deletion chromosome, compared with the sequence of the inter-γ region and $^{A}\gamma$IVS-2 of chromosome B (clone 51.1) of Slightom et al, 1980 (12). The nucleotides at the breakpoint are numbered as in Shen et al., 1981 (13). Computer-assisted homology searches showed no significant homology around the breakpoint.

for genetic recombination (14). To determine whether this (purine-pyrimidine) tract was at the endpoint of the 2.5 kb deletion, the 10.5 kb fraction of a Bgl II genomic digest was cloned into the bacteriophage L47 and sequencing of the insert performed from the Eco R1 site at position 5135, without further subcloning. The breakpoints are at position 5305 and 7827 of the sequence of Shen et al (13), a deletion of 2522 base pairs (Table 2). It is not possible to determine which breakpoint the C at this position originated from. However, the 3' breakpoint is 300 bp upstream of the purine-pyrimidine run, suggesting that this tract was not involved in the generation of the deletion. Computer-assisted homology searches revealed no apparent homologies in the DNA sequence for 50 bp either side of the breakpoints, indicating that the deletion results from an illegitimate recombination event.

DISCUSSION

A DNA survey of the γ-globin gene region of over 2100 chromosomes from healthy Melanesians has revealed a single deletion due to an illegitimate recombination event. Surveys of this region of β^{A} chromosomes in other populations, mainly as part of haplotype studies, also suggest that such silent rearrangements are rare (15,16). Similarly, the finding of just three chromosomes with a novel Bgl II restriction enzyme site polymorphism, after screening of all samples with this enzyme, suggests that such "private" (population-specific) polymorphisms are also infrequent.

Various models have been proposed to explain the effects of deletions within the β-globin complex on the fetal to adult globin switch. The most popular has been that a regulatory sequence between the γ and δ genes might

switch off fetal Hb synthesis about the time of birth (17). Detailed analysis of various Hereditary Persistence of Fetal Haemoglobin (HPFH) and δβ-thalassaemia deletions which have their 5' ends in this region suggested that this control region might be localized to a pair of Alu I repeat elements 5' to the δ gene (18). However, the recent description of individuals with a 6.5 kb deletion which includes this region who do not show the phenotype of HPFH is very difficult to reconcile with this hypothesis (19). An alternative proposal (20) is that sequences, such as enhancers (21) or repressors (22), at the 3' end of large β-globin complex deletions, which are brought into close proximity to the γ-genes by the deletion may be important in determining the level of Hb F production.

The lack of raised Hb F in this Melanesian deletion leads us to advocate another factor which may be of importance in determining the extent to which a deletion affects the γ→β switch. It would appear that small deletions in this complex produce little or no interference with switching whereas deletions above a certain size do. The 2.5 kb deletion described here and the 5 kb deletion which produces a single γ gene (5,6) do not prevent complete down regulation of the γ genes. Similarly two small deletions at the 5' and 3' ends of the β globin gene (23,24) behave like typical β thalassaemia point mutations. The slightly larger 6.5 kb Greek deletion (19) produces either normal or slightly raised Hb F levels in heterozygotes (range 0.3-5.6%, mean 2.5%, J.S. Wainscoat personal communication). Deletion of the 7.5 kb between the δ and β genes to produce Hb Lepore produces a modest elevation in Hb F (1-5% in heterozygotes, 25) whereas the 10 kb Sicilian and Dutch deletions lead to more significant Hb F production (4-12% in heterozygotes) (26,27). Hb Kenya due to a large 22 kb deletion between the $^{A}\gamma$ and β genes is associated with HPFH (28). Although these observations do not explain the difference in Hb F production between large deletions producing δβ thalassaemia and HPFH, they do suggest that deletion of small (<6 kb) segments of DNA in most of the β gene cluster will not significantly alter switching. Insertion of an additional 5 kb of DNA to produce a triplicated γ gene chromosome also does not prevent normal switching (5,7). Although any molecular interpretation of this observation is of course speculative, it may be that small but not large alterations in domain or DNA loop size are tolerable. Alternatively the lack of effect of most deletions within the cluster might be seen as support for the importance of newly-apposed regulatory sequences in large deletions (20).

In view of the uncertainty which still exists about the nature, location and, perhaps, existence of important switch-controlling regions within the β-

complex, it is clearly important to continue defining the position, size and phenotypic effects of further deletions. Although those which increase Hb F production are probably selected and, therefore, more common as well as easier to detect phenotypically, it may be that silent deletions in the complex will eventually prove equally informative.

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References

1. Collins, F.S. and Weissman, S.M. (1984). Prog. Nucleic Acids Res. Mol. Biol., 31, 315-348.
2. Karlsson, S. and Nienhuis, A.W. (1985). Ann. Rev. Biochem., 54, 1071-1108.
3. Weatherall, D.J. and Clegg, J.B. (1981). The Thalassaemia Syndromes, Blackwell Scientific, Oxford, 3rd Ed.
4. Bowden, D.K., Hill, A.V.S., Higgs, D.R., Weatherall, D.J. and Clegg, J.B. (1985). Lancet ii, 1025-1028.
5. Hill, A.V.S., Bowden, D.K., Weatherall, D.J. and Clegg, J.B. (1986). Blood, In Press.
6. Sukumaran, P.D., Nakatsuji, T., Gardiner, M.B., Reese, A.L., Gilman, J.G. and Huisman, T.H.J. (1983). Nucleic Acids Res., 11, 4635-4643.
7. Trent, R.J., Bowden, D.K., Old, J.M., Wainscoat, J.S., Clegg, J.B. and Weatherall, D.J. (1981). Nucleic Acids Res., 9, 6723-6733.
8. Old, J.M. and Higgs, D.R. (1983). In The Thalassaemias, ed. Weatherall, D.J., Methods in Haematology, 6, 74-102.
9. Wood, W.G. (1983). In The Thalassaemias, ed. Weatherall, D.J. Methods in Haematology, 6, 31-53.
10. Maniatis, T., Fritsch, E.F., Sambrook, J. (1982). Molecular Cloning. Cold Spring Harbor Laboratory, New York.
11. Maxam, A.M. and Gilbert, W. (1977). Proc. Natl. Acad. Sci. USA, 74, 560-564.
12. Slightom, J.L., Blechl, A.E. and Smithies, O. (1980). Cell, 21, 627-658.
13. Shen, S., Slightom, J.L. and Smithies, O. (1981). Cell, 26, 191-203.
14. Stoeckert, C.J., Collins, F.S. and Weissman, S.M. (1984). Nucleic Acids Res., 12, 4469-4479.
15. Antonarakis, S.E., Kazazian, H.H. Jr. and Orkin, S.H. (1985). Hum. Genet., 69, 1-14.
16. Wainscoat, J.S., Hill, A.V.S., Boyce, A.J., Flint, J., Hernandez, M., Thein, S.L., Old, J.M., Lynch, J.R., Falusi, Y., Weatherall, D.J. and Clegg, J.B. (1986). Nature, 319, 491-493.

17. Huisman, T.H.J., Schroeder, W.A., Efremov, G.D., Duma, H., Mladenovskil, B., Hyman, C.B., Rachmilewitz, E.A., Bouver, N., Miller, A., Brodie, A., Shelton, J.R., Shelton, J.B. and Apell, G. (1974). Ann. N.Y. Acad. Sci., 232, 107-124.
18. Ottolenghi, S. and Giglioni, B. (1982). Nature, 300, 770-771.
19. Wainscoat, J.S., Thein, S.L., Wood, W.G., Weatherall, D.J., Metaxotou-Mavromati, A., Tzotos, S., Kanavakis, E. and Kattamis, C. (1985). Ann. N.Y. Acad. Sci., 445, 20-27.
20. Tuan, D., Feingold, E., Newman, M., Weissman, S.M. and Forget, B.G. (1983). Proc. Natl. Acad. Sci., USA, 80, 6937-6941.
21. Feingold, E., Collins, F.S., Metherall, J.E., Stoeckert, C.J., Weissman, S.M. and Forget, B.G. (1985). Ann. N.Y. Acad. Sci., 445, 159-169.
22. Mager, D.L. and Henthorn, P.S. (1984). Proc. Natl. Acad. Sci., USA, 81, 7510-7514.
23. Spritz, R.A., Orkin, S.H. (1982). Nucleic Acids Res., 10, 8025-8029.
24. Padanilam, B.P., Felice, A.E., Huisman, T.H.J. (1984). Blood, 64, 941-944.
25. Wood, W.G., Clegg, J.B., Weatherall, D.J. (1979). Br. J. Haematol., 43, 509-520.
26. Bernards, R., Kooter, J.M., Flavell, R.A. (1979). Gene, 6, 265-280.
27. Gilman, J.G., Huisman, T.H.J., Abels, J. (1984). Br. J. Haematol., 56, 339-348.
28. Ojwang, P.J., Nakatsuji, T., Gardiner, M.B., Reese, A.L., Gilman, J.G., Huisman, T.H.J. (1983). Hemoglobin 7, 115-123.